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Supplemental Data

Germline Mutation in *ATR* in Autosomal-Dominant Oropharyngeal Cancer Syndrome

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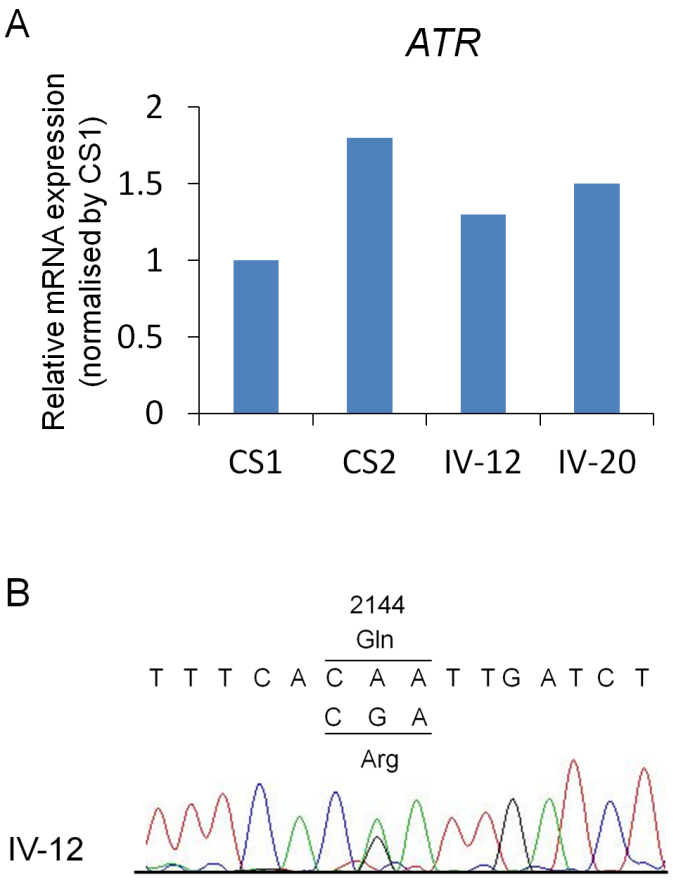

Figure S1. Quantitative Real-Time PCR Analysis and Sequence Analysis of *ATR* mRNA in the Skin Samples

(A) Relative quantification of *ATR* mRNA expression. CS1 and CS2 indicate control skin samples from two healthy donors; IV-12 and IV-20 are skin samples taken from affected individuals in Figure 1A. The gene expression was normalized against the expression of 18srRNA and expressed relative to CS1.

(B) Nucleotide sequencing of cDNA from skin mRNA in affected individual. IV-12 reveals a heterozygous missense mutation, c.6431A>G (p.Gln2144Arg) in *ATR*. This mutation was also demonstrated in the skin-derived cDNA from individuals IV-20 (data not shown).

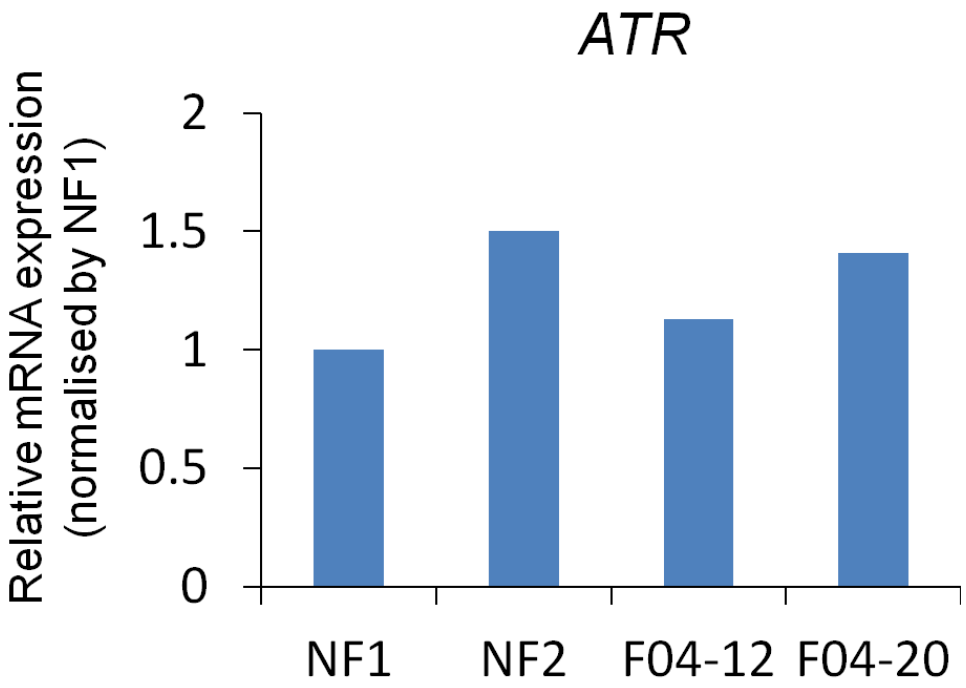

Figure S2. Relative Quantification of *ATR* mRNA Expression in Cultured Fibroblasts

NF1 and NF2 indicate control fibroblasts from two healthy donors; F04-12 and F04-20 are fibroblasts from carriers IV-12 and IV-20 in Figure 1A, respectively. The gene expression was normalized against expression of 18srRNA and expressed relative to NF1.

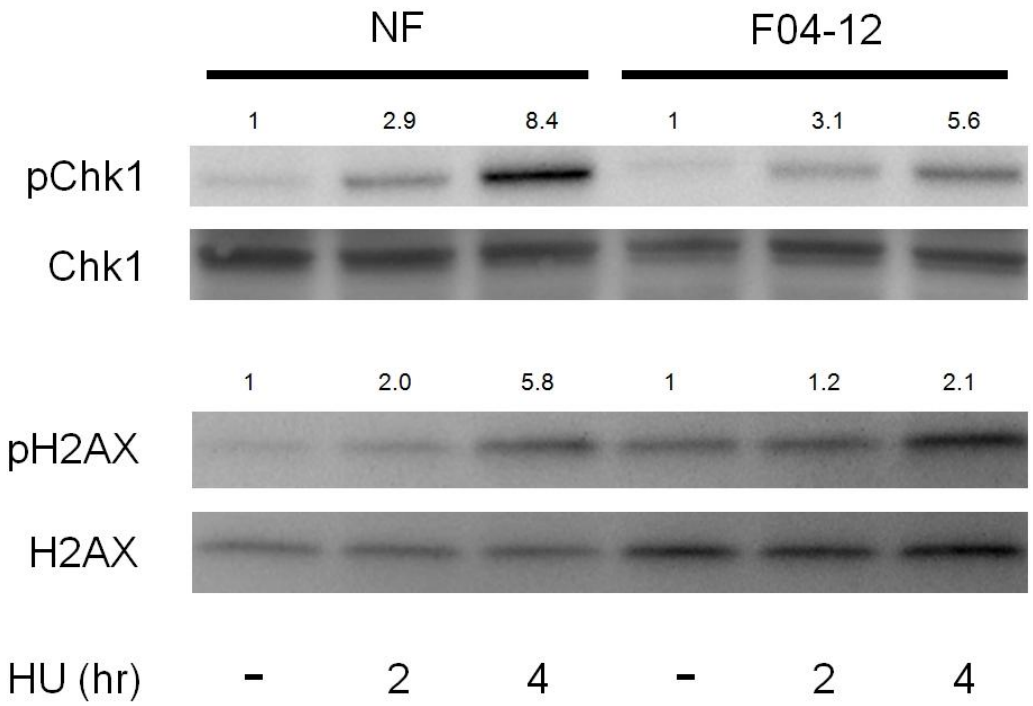

Figure S3. Western Blot for Phosphorylation of Chk1 and H2AX in Cultured Fibroblasts (Affected/Control) Pre- and Postactivation of ATR by Hydroxyurea (HU) Exposure for 2 or 4 Hours

The control fibroblasts (NF) and the affected individual fibroblasts (F04-12) show the same pattern in the phosphorylation of Chk1 and H2AX. The number immediately above each band in the western blot indicates the relative intensity of the corresponding band. The antibody for pChk1 (Ser345) (133D3) (#2348), H2AX (#2595), and phosphor-Histone H2AX (Ser139) (20E3) (#9718) were purchased from Cell Signaling; Chk1 (G-4) (sc8408) was purchased from Santa Cruz.

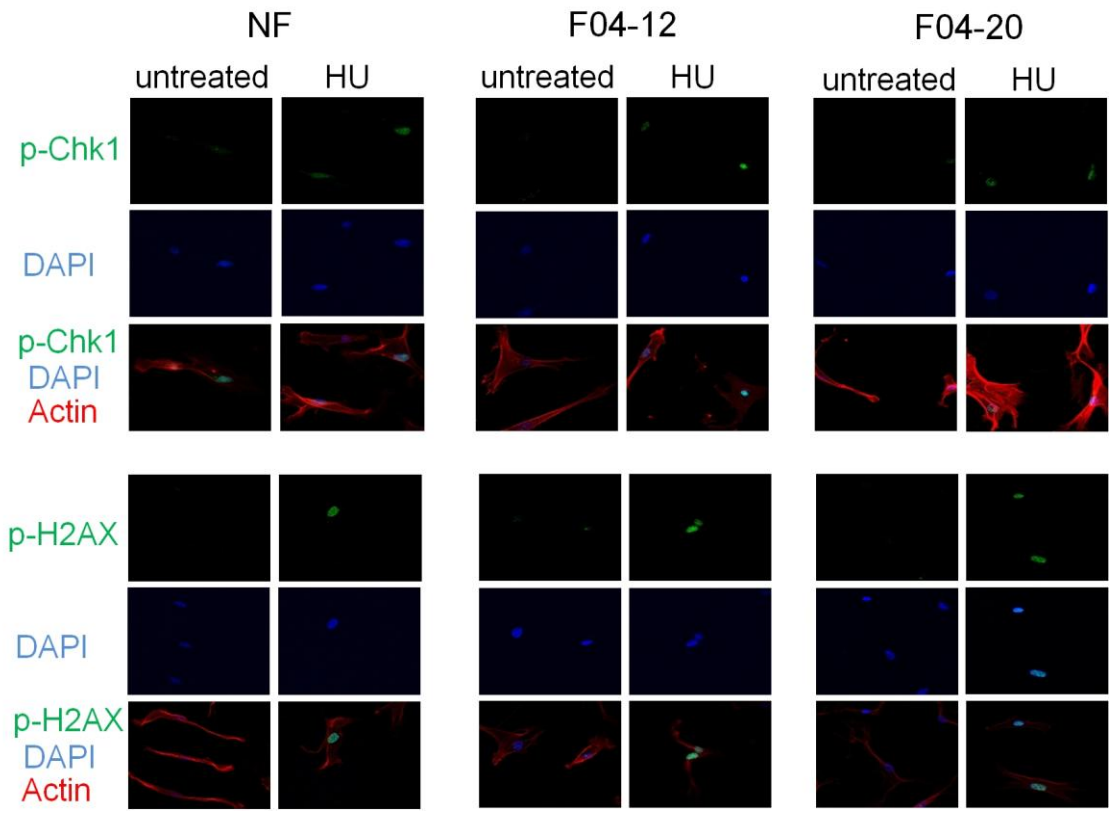

Figure S4. Immunocytochemistry for p-Chk1 and p-H2AX in Fibroblasts (Affected Individuals, F04-12 and F04-20; Unaffected Control, NF) Pre- and Postactivation of ATR by Hydroxyurea (HU) Exposure for 2 hours

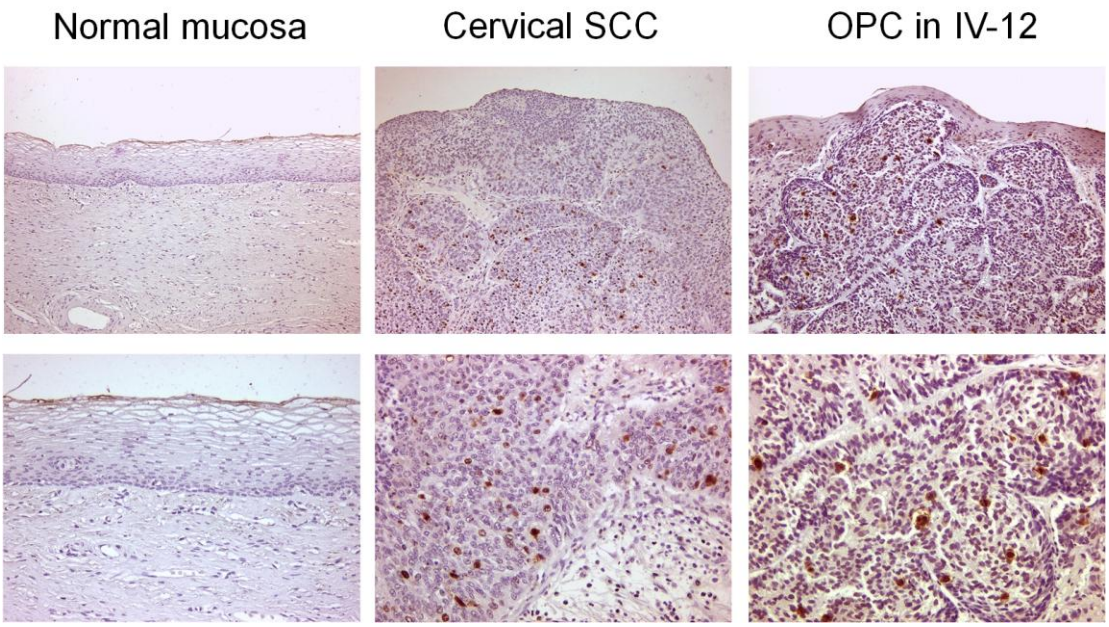

Figure S5. Immunohistochemistory for ATR in Biopsy Specimens Taken from Normal Cervical Mucosa (Normal Mucosa), Cervical Squamous Cell Carcinoma (Cervical SCC), and OPC SCC from Subject IV-12

Normal cervical mucosa does not show any ATR labeling, but some epithelial nuclei in mitotic cells in cervical SCC do show positive ATR immunoreactivity. The OPC SCC in IV-12 also shows a similar pattern to cervical SCC with some positive nuclear staining for ATR in the tumor. Upper panels: original magnification X100. Lower panels: original magnification X200. Anti-ATR antibody (ab110883) was purchased from Abcam (Cambridge, UK).

Table S1. Clinical Details of Affected Individuals with this Autosomal-Dominant Disorder

Patient number	age, height and weight	Past medical history	Age at appearance of skin lesions	Dentition	skin	Hair	Nails	Other
Female III:7	40 years 5'6" 200lbs	None listed	DNA	Broken and missing teeth, several caries, thin enamel	Telangiectases and hyperpigmentation on the face, submentum, extremities, buttocks, and flanks	Thin lateral eyebrows. Alopecia in areas of telangiectases	Mild longitudinal ridging Thickened yellow toenails	N
Male III:18	56 years 5'7" 170lbs	Heart disease, Lung disease, Arthritis, Hernia repair, Gallbladder surgery, CABG, several knee surgeries	2mos	Edentulous with full dentures	Telangiectases and hyper/hypo pigmentation on the face with several actinic keratoses and solar lentigens. Teleangiectasia and hyper/hypo pigmentation also present on extremities, and buttocks.	Thin lateral eyebrows. Alopecia in areas of telangiectases	Longitudinal ridging of nails Onychomycosis of toenails	Palms and soles: Slightly thickend and rough
Female IV:2	8.5 years 4'5" 70lbs	Heart murmur	18mos	Pegged frontal incisors, thin enamel	Telangiectases and hyperpigmentation on the face, extremities and flanks.	Thin lateral eyebrows. Alopecia in areas of teleangiectasia	N	N
Male IV:3	4 years 40.5lbs	None listed	Infancy	Bifid lower incisor, caries, thin enamel	Telangiectases and hyperpigmentation on cheeks, extremities, ears, flanks, lateral arms, legs, and buttocks	Thin lateral eyebrows. Alopecia in areas of telangiectases	Pterygium left index nail Dystrophic 5th toenail left foot	N
Male IV:6	40 years 5'11" 140lbs	None listed	Unknown	Edentulous with full dentures	Telangiectases and hyperpigmentation on face, neck, extremities, and buttocks	Thin lateral eyebrows. Alopecia in areas of telangiectases	Longitudinal ridging, cuticular overgrowth, thumbnails appear somewhat clubbed	Palms and soles: Xerotic palms Ears: Cataracts per history Facial features: Mild beak-like facies and large ears
Female IV:12	35 years 4'11" (short stature) 92lbs	Hypothylid, Removal of tonsils and adenoids	Birth	Missing teeth and prominent caries	Telangiectases and hyperpigmentation on the cheeks, chin, submentum, ears, extremities, buttocks and flanks	Thin lateral eyebrows. Alopecia in areas of telangiectases	N	Facial features: Beak nose
Female IV:18	25 years 5'2" 105lbs	None listed	unknown	Loss of multiple teeth, several caries, yellowing and thinning of enamel	Telangiectases on face, extremities, and buttocks	Thin lateral eyebrows. Alopecia in areas of telangiectases	Few longitudinal ridges	Facial features: Somewhat narrow/beak nose
Female IV:20	30 years 5'4" 120lbs	Kidney stones Gallbladder surgery	6mos	Loss of teeth, missing mostly the molars, remaining teeth appear yellow and decayed	Telangiectases and hyperpigmentation on cheeks, extremities, buttocks, post hips, livedo on legs	Thin lateral eyebrows. Alopecia in areas of teleangiectasia	Unable to assess (wearing acrylics) Pt reports they are thin and crack easily	N
Female V:1	9 years 90lbs	eartubes	unknown	Multiple caries	Telangiectases on the face, upper extremities, flanks, lower extremities and buttocks	Thin lateral eyebrows. Alopecia in areas of telangiectases	N	Eyes: Contact lenses
Female V:2	6 years 60lbs	None listed	1mo	Front incisors pegged; several dental caries noted; decreased enamel	Telangiectases on the ears, cheeks, chin, submentum, upper chest, flanks , extremities, and buttocks	Thin lateral eyebrows. Alopecia in areas of telangiectases	N	N
Male V:8	8 years 48lbs	Heart murmur, Growth hormone problems	2mos	Spacers and palatal expander in place, caries, thin enamel	Telangiectases on the cheeks, ears, extremeties, and buttocks	Alopecia in areas of telangiectases	N	N
Female V:10	12 years 5'1" 83lbs	Eartubes	6mos	Rare cavity	Telangiectases on the face, primarily the cheeks, extremeties, buttocks and flanks	Thin lateral eyebrows. Alopecia in areas of teleangiectasia	Onycholysis and subungual debris left toenails	N

DNA: did not answer the question. N: no/normal.

Table S2. Cancer Surveys of Affected Individuals with this Autosomal-Dominant Disorder

Patient number	Age	History of smoking	History of Alcohol	History of cancer (age at appearance)	Site of oropharyngeal cancer
Male II:2		DNA	DNA	Oropharyngeal cancer (DNA)	
Female II:6		Smoker of unknown duration	DNA	Oropharyngeal cancer (DNA)	
Male II:7		N	DNA	Oropharyngeal cancer (DNA)	
Female II:12		N	DNA	Oropharyngeal cancer (DNA)	
Male III:2		Cigarettes 1ppd 20+ years	DNA	Oropharyngeal cancer (DNA)	
Female III:3		N	DNA	DNA	
Female III:7	40 years	N	DNA	DNA	
Male III:9		N	DNA	Oropharyngeal cancer (DNA)	
Female III:12	63 years	N	N	Oropharyngeal squamous cell carcinoma (63 years) Squamous cell carcinoma of the skin (DNA)	
Female III:15		Cigarettes 1-2ppd 30-40 years	DNA	Oropharyngeal cancer (DNA)	
Male III:18	56 years	N	DNA	Non-melanoma skin cancers (DNA)	
Male III:22		N	DNA	DNA	
Female IV:2	8.5 years	N	N	N	
Male IV:3	4 years	N	N	N	
Male IV:6	40 years	Chew tobacco	DNA	N	
Female IV:8	35 years	DNA	DNA	Breast cancer (DNA)	
Female IV:12	35 years	N	DNA	Oropharyngeal squamous cell carcinoma (34 years) Non-melanoma skin cancer on the arm (DNA)	Poorly differentiated SCC of the posterior pharyngeal wall (HPV negative)
Female IV:18	25 years	Cigarettes 1ppd 12 years	DNA	N	
Female IV:20	30 years	Cigarettes 0.5ppd 10 years	One glass wine per day 10years	Oropharyngeal squamous cell carcinoma (30years) Cervical cancer (28years)	Moderately differentiated SCC of the soft palate and left tonsil (HPV negative)
Female V:1	9 years	N	N	N	
Female V:2	6 years	N	N	N	
Male V:8	8 years	N	N	N	
Female V:10	12 years	N	N	N	

DNA: did not answer the question. N: no/normal.

Table S3. Candidate Genes Sequenced in this Study

	Gene symbol	MIM number
1	*ASTE1*	
2	*NEK11*	609779
3	*NUDT16*	
4	*MRPL3*	607118
5	*CPNE4*	604208
6	*ACPP*	171790
7	*CCRL1*	606065
8	*CDV3*	
9	*TOPBP1*	607760
10	*SRPRB*	
11	*RAB6A*	179513
12	*SLCO2A1*	601460
13	*RYK*	600524
14	*AMOTL2*	
15	*ANAPC13*	
16	*CEP63*	
17	*EPHB1*	600600
18	*PPP2R3A*	604944
19	*PCCB*	232050
20	*STAG1*	604358
21	*TMEM22*	
22	*NCK1*	600508
23	*CLDN18*	609210
24	*DZIP1L*	
25	*DBR1*	607024
26	*MRAS*	608435
27	*CEP70*	614310
28	*FAIM*	
29	*PIK3CB*	602925
30	*RBP2*	180280
31	*RBP1*	180260
32	*NMNAT3*	608702
33	*RASA2*	601589
34	*RNF7*	603863
35	*TFDP2*	602160
36	*XRN1*	607994
37	*ATR*	601215
38	*PLS1*	602734
39	*CHST2*	603798
40	*PLSCR2*	
41	*ZIC4*	608948
42	*ZIC1*	600470